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Chlordimeform Produces Profound, Selective, and Transient Changes in Visual Evoked Potentials of Hooded Rats

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16. ABSTRACT

Rat visual function was tested after acute exposure to chlordimeform (CDM), a formamidine insecticide/acaricide. Adult male Long-Evans rats were surgically implanted with epidural recording electrodes overlying visual cortex and tested 1 week later. Pattern reversal-evoked potentials (PREPs), flash-evoked potentials (FEPs), and FEP recovery ratios were measured after acute CDM administration. Averaged recordings obtained during 200 reversals of a black-and-white square wave grating comprised the PREPs, and those obtained during 128 paired strobe lamp flashes comprised the FEPs. In the first study, which exmained dose-response relationships, i.p. injections of 0 (saline), 5, 15, or 40 mg/kg CDM-HCl were administered 30 min prior to testing. The PREP amplitudes showed large dose-related changes in the CDM-treated rats. PREP N1P1 and P1N3 peak-to-peak amplitudes increased more than 200% in the 40 mg/kg group. In contrast, FEP amplitudes and FEP recovery ratios were unchanged by CDM. Both PREP and FEP peak latencies were increased by CDM in dose-related fashions. In the second study, which examined the time course of CDM action, PREPs and paired-pulse FEPs were recorded 3, 6, and 24 h after dosage with either 0 or 40 mg/kg CDM. All evoked potential changes were large at 3 and 6 h, but had returned to control values by 24 h. In summary, acute exposure to CDM temporarily increased both the amplitude and latency of PREPs, but only the latency of FEPs.

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Chlordimeform Produces Profound, Selective, and Transient Changes in Visual Evoked Potentials of Hooded Rats

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Rat visual function was tested after acute exposure to chlordimeform (CDM), a formamidine insecticide/acaricide. Adult male Long–Evans rats were surgically implanted with epidural recording electrodes overlying visual cortex and tested 1 week later. Pattern reversal-evoked potentials (PREPs), flash-evoked potentials (FEPs), and FEP recovery ratios were measured after acute CDM administration. Averaged recordings obtained during 200 reversals of a black-and-white square wave grating comprised the PREPs, and those obtained during 128 paired strobe lamp flashes comprised the FEPs. In the first study, which examined dose–response relationships, i.p. injections of 0 (saline), 5, 15, or 40 mg/kg CDM-HCl were administered 30 min prior to testing. The PREP amplitudes showed large dose-related changes in the CDM-treated rats. PREP N1P1 and P1N3 peak-to-peak amplitudes increased more than 200% in the 40 mg/kg group. In contrast, FEP amplitudes and FEP recovery ratios were unchanged by CDM. Both PREP and FEP peak latencies were increased by CDM in dose-related fashions. In the second study, which examined the time course of CDM action, PREPs and paired-pulse FEPs were recorded 3, 6, and 24 h after dosage with either 0 or 40 mg/kg CDM. All evoked potential changes were large at 3 and 6 h, but had returned to control values by 24 h. In summary, acute exposure to CDM temporarily increased both the amplitude and latency of PREPs, but only the latency of FEPs. © 1984 Academic Press, Inc.

Abbreviations: CDM—chlordimeform; FEP, PREP—flash-, pattern reversal-evoked potential.

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INTRODUCTION

Chlordimeform (CDM) is a formamidine insecticide and acaricide (Fig. 1). Many biological actions of CDM have been reported including monoamine oxidase or N-acetylation inhibition (2–6, 21, 28), local anesthetic-like activity (9, 25, 34), disrupted neuromuscular transmission via depression of end-plate sensitivity (41), uncoupling of oxidative phosphorylation (1), and prostaglandin synthesis inhibition (43). However, many of those effects were obtained only at high *in vitro* concentrations, and/or did not correlate well with *in vivo* intoxication (16, 31). Growing evidence points toward disruption of monoaminergic neurotransmission as the possible mechanism of pesticidal action (14, 18, 30, 37, 38). This evidence, recently reviewed by Hollingworth and Lund (17), indicates that neurobehavioral consequences of CDM exposure are likely.

In mammalian species, CDM produces behavioral changes in a variety of paradigms including measures of locomotor activity, flavor aversion, schedule-controlled behavior, and social interactions (24, 26, 27, 32, 35, 36, 42). Mammalian neurophysiological studies, which may indicate neural systems sensitive to CDM, have been described only once (9). Mammalian visual function is dependent, in part, on monoaminergic neurotransmission (7), and therefore may be vulnerable to CDM exposure. Flash-evoked potentials (FEPs) and pattern reversal-evoked potentials (PREPs) of hooded rats are disrupted by monoaminergic manipulations (12, 33), and reflect the general functional integrity of the visual system. Therefore, a series of studies was conducted in which FEPs and PREPs were recorded in hooded rats exposed to CDM.

MATERIALS AND METHODS

Animals. Adult male Long–Evans hooded rats (Charles River) weighing 250 to 400 g at the time of surgery were used. The rats were individually housed in plastic cages on wood chip bedding with *ad libitum* access to food and water, and were maintained on a 12-h light:12-h dark cycle (lights on at 0600 h).

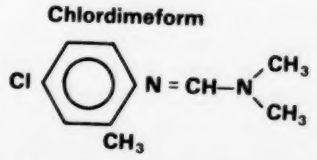

FIG. 1. Structure of chlordimeform (CDM).

Surgery. Prior to each experiment, the rats were anesthetized with Chloropent (3.5 ml/kg, i.p., Fort Dodge Labs, Inc.) and placed in a stereotaxic frame for chronic electrode implantation. Stainless-steel screw electrodes (00–90 × 1/16 in.) were threaded into the skull in several places: 2 mm anterior to bregma and 2 mm left and right of midline for the ground and reference electrodes, respectively, and 6 mm posterior to bregma and either 3 or 4 mm lateral to midline for the FEP and PREP visual cortex electrodes. The electrodes were attached to an Amphenol connector and the entire assembly encased in dental acrylic. Each rat received Nitrofurazone as a topical antibiotic before the wound was sutured, and 100,000 U penicillin G i.m. postoperatively. Experiments commenced no less than 1 week postoperatively.

Doses. Chlordimeform-HCl (97% pure from NOR-AM Agricultural Products, Naperville, Ill.) was dissolved in isotonic saline and administered in single i.p. injections in a volume of 1 ml/kg body weight. Doses of 0, 5, 15 and 40 mg/kg, measured as the salt, were chosen to encompass the range over which most behavioral studies have been made (36), although each experiment did not include every dose level. The dosages used were well below the lethal range. Reported i.p. LD_{50} values for rats include 123 mg/kg (40), 238 mg/kg (39), and between 75 and 125 mg/kg (6). Fresh solutions were made daily and stored in the dark until use to delay photo-decomposition of the CDM (15).

Testing Procedures. The FEPs were recorded from awake rats placed in a small chamber with mirrors on three walls and the strobe lamp of a Grass Model PS-22 photostimulator on the fourth wall. The stimulator was set at an intensity of 16, which provides a 10-μs flash of approximately 4.53 $\times 10^7$ lx peak power. The FEPs were recorded in a paired-pulse procedure in which two flashes were presented with an interflash interval of 250 ms.

The PREPs were recorded from awake rats restrained in a harness (Alice King Chatham Medical Arts) and placed 7 cm in front of the TV screen of a Grass Model VPG pattern generator. Horizontal black and white bars (a square wave grating) (0.2 cycles per degree visual angle, 99% contrast, 30 cd/m^2, 69° × 74° field size), displayed on the screen were reversed black for white so that there was no overall change in stimulus intensity. The flash pair and pattern shift rates were both 0.5 Hz. More detailed descriptions of the methodology may be found elsewhere (8, 10).

Electrocortical activity from the implanted electrodes was amplified (×10,000), filtered (0.8 to 100 Hz for the PREP and 0.8 to 1000 Hz for the FEP), A/D converted at 2500 Hz, and passed to a PDP 11/70 computer for signal averaging and data storage. Averaged evoked potentials were computed for each rat from 128 trials for the FEP and 200 trials for the PREP. The computer displayed waveforms on a terminal and calculated

peak latencies and peak-to-peak amplitudes from the operator-selected points. Group average evoked potential waveforms were computed from the stored data and are presented here to illustrate group trends. All statistical analyses, however, were carried out on individual rat values.

Experiment 1 Protocol. To determine dose–response relationships of CDM on the PREP and FEP, rats were randomly divided into four groups which received either 0 ($N = 17$), 5 ($N = 17$), 15 ($N = 14$), or 40 ($N = 19$) mg/kg CDM. The FEPs and PREPs were recorded from each rat in a counterbalanced order beginning 30 min after treatment.

Experiment 2 Protocol. The time course of CDM action on the FEP and PREP was examined at 3, 6, and 24 h after administering either 0 ($N = 11$) or 40 ($N = 16$) mg/kg CDM. The presentation order of FEPs and PREPs was counterbalanced.

Statistical Procedures. Each experiment used a separate, independent group of rats and was analyzed individually. Within each experiment, the statistical procedures were designed to protect against increasing the frequency of type I errors while making multiple measurements on the same rats (29). Data analysis of each experiment began with a series of multivariate analyses of variance (MANOVAs) containing related sets of dependent variables. The criterion probability defining statistical significance (0.05) was divided by the number of MANOVAs for each experiment according to the Bonferroni correction procedure. Statistically significant MANOVAs were followed with ANOVAs on each of the included dependent variables, again dividing the alpha level by the number of ANOVAs within each MANOVA. Significant ANOVAs were in turn followed by Duncan's multiple-range or Tukey's tests to determine which treatment contributed to the differences for that particular dependent variable.

Five MANOVAs were run for experiment 1, one each for PREP latencies, PREP amplitudes, FEP latencies from the first of the paired flashes, FEP amplitudes from the first of the paired flashes, and FEP recovery ratios expressed as the latency or amplitude of the response to the second flash divided by the analogous value of the response to the first flash. For experiment 2, four MANOVAs were run. They were identical to those from experiment 1 with two exceptions, (a) the FEP amplitude MANOVA was omitted because of unambiguous negative findings in experiment 1, and (b) multivariate repeated measures procedures were used because of the multiple time point recordings from each rat.

RESULTS

When handled, rats receiving 40 mg/kg CDM vocalized, clung to labcoats and other objects, and exhibited tremors (myotonia). These signs were more

moderate in rats which received 15 mg/kg, and were generally absent in rats receiving 5 mg/kg. Pupillary dilation was not noted under any condition.

Group-averaged PREP and paired-pulse FEP waveforms from experiment 1 are presented in Fig. 2. The control group PREP shows five readily identifiable peaks as described elsewhere (8). The CDM treatment produced a dose-related enlargement of PREP peaks P1 and N3. PREP peaks N2 and P2 were frequently not detectable in the waveforms of the CDM-treated rats. The FEP group-averaged waveform of the control rats, and those from the groups of CDM-treated rats all contained the major peaks normally found in awake hooded rats (13). In the FEP group-averaged waveforms, there were no amplitude changes comparable to those produced by CDM in the PREP. In contrast to the selective amplitude changes induced by CDM, the latency of both PREP and FEP peaks increased as a

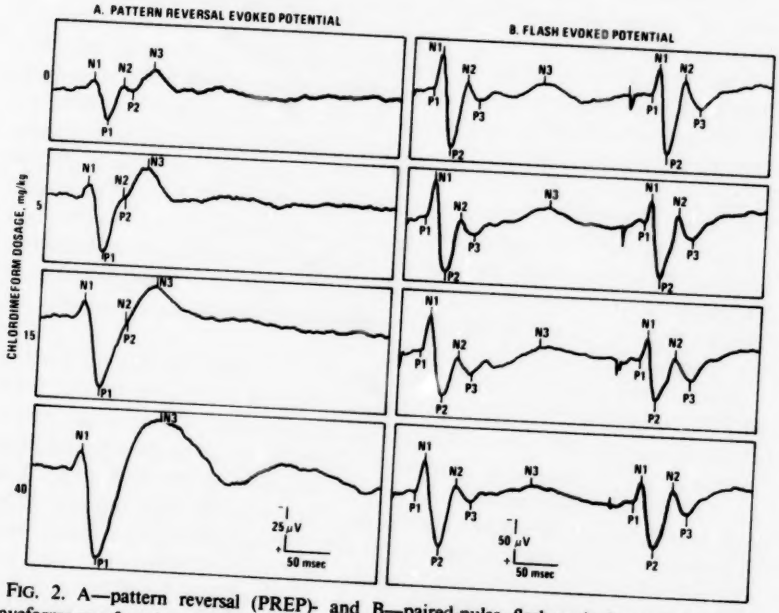

FIG. 2. A—pattern reversal (PREP)- and B—paired-pulse flash-evoked potential (FEP) waveforms as a function of CDM dose. Individual evoked potential values were averaged across $N = 17$, $N = 17$, $N = 14$, and $N = 19$ rats to obtain the group averaged waveforms for doses 0, 5, 15, and 40 mg/kg, respectively. Each rat received only one dose. The PREP and FEP were recorded in a counterbalanced order. Treatment with CDM produced dose-related increases in PREP N1P1 and P1N3 amplitudes and an apparent loss of N2P2. No such amplitude changes occurred in FEPs. Peak latencies in both the PREP and FEP increase as a function of dose. FEP recovery ratio was unchanged by CDM.

function of dose. Recovery of the FEP, evaluated by comparison of the second waveform of each pair to the first, was unchanged by CDM.

The rats receiving CDM showed dose-related changes in PREP peak-to-peak amplitudes (Fig. 3). The measurements for PREP peaks N2 and P2 cannot be considered highly reliable due to the difficulty of identifying those two peaks in the waveforms of treated rats, and are therefore not presented. As an exception, N2P2 amplitude is included in Fig. 3 because it could be scored as zero when absent. The MANOVA for PREP peak-to-peak amplitudes showed a significant effect of dose [$F(12, 131) = 3.53$, $P = 0.002$]. The individual amplitude ANOVA was significant for N1P1 [$F(3, 48) = 4.76$, $P = 0.005$]. Duncan's test indicated that the 40- and 15-mg/kg groups were different from controls, but the 5-mg/kg group was not. The mean N1P1 amplitudes of both the 15- and 40-mg/kg CDM groups were two to three times the control group value. The dose effect of N2P2 amplitude [$F(3, 60) = 3.18$, $P = 0.0299$] failed to meet the conservative criterion for statistical significance and could not be considered significant because the required P value after Bonferroni correction was $0.05 \div 4$

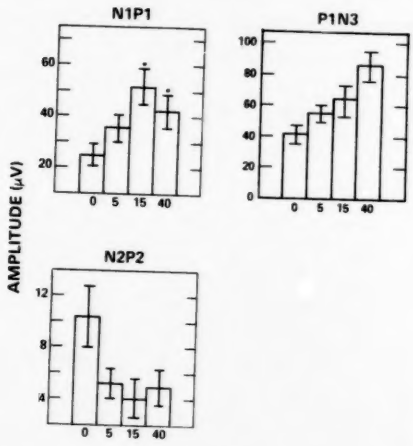

FIG. 3. PREP peak-to-peak amplitudes as a function of CDM dose. Amplitudes P1N2 and N2P2 could not be reliably identified in CDM-treated rats, and are therefore not presented. Instead, P1N3 amplitude was calculated, but was not included in the statistical analysis because of the *post hoc* nature of the measure. CDM treatment produced dose-related enlargements of N1P1 and P1N3, and a reduction of N2P2 which did not attain statistical significance. In this and subsequent figures, the plotted values represent $\bar{X} \pm SE$, and statistically significant treatment effects are indicated with an *.

= 0.0125. Because of the peak identification problem, a post hoc calculation of P1N3 amplitude was carried out (Fig. 3). The amplitude of P1N3 was increased by CDM in a dose-related fashion. For the 40-mg/kg CDM group, the mean P1N3 amplitude was approximately twice that of controls. P1N3 is more reliable than P1N2 or N2P3 as it can be scored in all PREPs, but it was not included in the statistical analysis of the experiment because its potential value was apparent only after the data were collected and scrutinized.

The latency of PREP peaks was increased by CDM exposure (Fig. 4). Among the PREP peaks, P1 appeared to be most sensitive to CDM action, for it increased monotonically with dose. Significant increases in PREP peak latencies were found in the MANOVA [$F(15, 128) = 15.04$, $P < 0.0001$] and in all individual peak ANOVAs [all Fs$(3, 48) > 17.08$, all Ps < 0.0001]. Duncan's test revealed that all groups differences were due to the 15- and 40-mg/kg groups. For the 5-mg/kg group, the trend toward increased latency in peak P1 was not statistically significant.

Unlike PREP amplitudes, the FEP amplitudes were unaffected by CDM exposure. The FEP peak-to-peak amplitude values were within the normal range for hooded rats (13) for all dose groups, and showed no apparent dose–effect trends (data not presented). The FEP amplitude MANOVA was not significant [$F(15, 143) = 0.90$, $P > 0.56$].

The FEP latencies were increased by CDM. Peak latencies for every measured FEP peak were greater in the 15- and 40-mg/kg groups than in controls (Fig. 5). Peaks P1, N1, P2, and N2 all showed monotonically

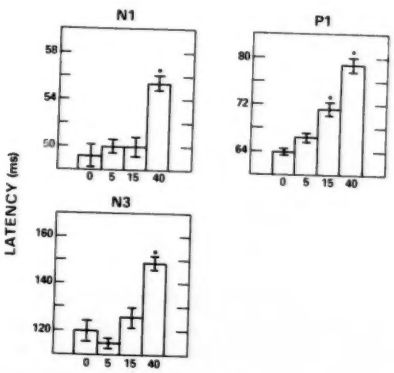

FIG. 4. PREP peak latencies as a function of CDM dose. CDM treatment prolonged all PREP peak latencies in a dose-related fashion.

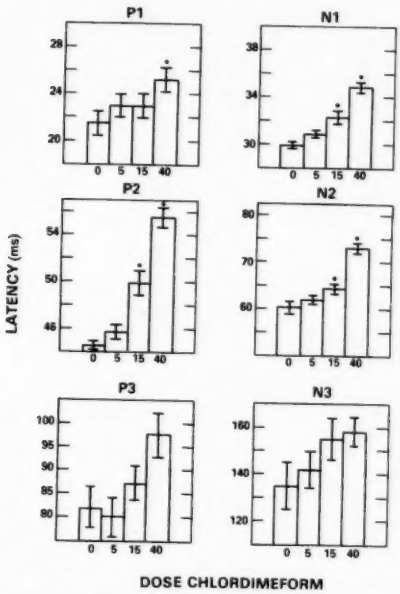

FIG. 5. FEP peak latencies as a function of CDM dose. Treatment with CDM significantly increased the latency of the earlier FEP peaks P1, N1, P2, and N2, but changes in P3 and N3 did not attain significance.

increasing latencies as a function of dose. The dose factor of the FEP peak latency MANOVA was statistically significant [$F(18, 140) = 9.64$, $P < 0.0002$]. The individual peak ANOVAs were significant for peaks P1, N1, P2, and N2 [all Fs$(3, 53) > 5.55$, all Ps < 0.002]. The ANOVA for peak P3 ($F(3, 53) = 3.37$, $P = 0.02$] cannot be considered statistically significant because a probability value of $0.05 \div 6 = 0.0083$ was required. Duncan's test indicated that no significant changes were produced by 5 mg/kg, that N1, P2, and N2 latencies were significantly increased by 15 and 40 mg/kg, and that P1 latency was significantly increased by only 40 mg/kg.

The FEP recovery ratios were not altered by CDM exposure [MANOVA $F(27, 131) = 1.46$, $P = 0.0828$]. Neither the amplitude nor latency recovery ratios showed any trend toward alteration as a function of dose (data not shown).

The results of the time-course study (experiment 2) are presented in Figs. 6 and 7. Only a few variables are plotted because the time course was fairly uniform across measurements. The PREP amplitudes and latencies, and FEP latencies, were all increased 3 and 6 h after CDM exposure. The values

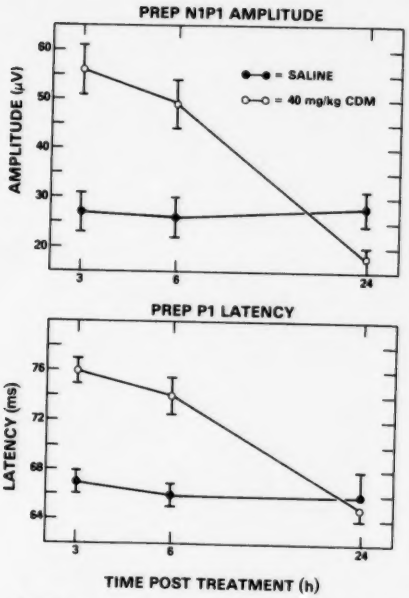

FIG. 6. Time course of CDM action on selected PREP latency and amplitude values. Other measured variables showed a similar temporal pattern. The CDM effects, evident at 3 h, were somewhat less at 6 h and returned to normal at 24 h.

were generally somewhat lower at 6 h than at 3 h, and had consistently returned to control values by 24 h. At 3 and 6 h after administration, the mean PREP N1P1 amplitude of the 40-mg/kg CDM group was two to three times control values, consistent with the results of experiment 1. In the PREP amplitude MANOVA, the main effect of dose was not significant [$F(4, 26) = 2.24$, $P = 0.0922$], but significant time [$F(8, 96) = 16.83$, $P < 0.0001$] and dose × time [$F(8, 96) = 10.70$, $P < 0.0001$] effects were found. In the PREP latency MANOVA, significant main effects of dose [$F(5, 25) = 5.56$, $P = 0.0014$] and time [$F(10, 94) = 5.70$, $P < 0.0001$], and a significant dose × time interaction [$F(10, 94) = 4.93$, $P < 0.0001$] were detected. The ANOVA dose × time interaction was significant for peak P1 latency and for N1P1 amplitude [both Fs$(2, 52) > 11.81$, both Ps < 0.0001]. The FEP latency MANOVA showed significant dose [$F(6, 27) = 4.61$, $P < 0.002$], time [$F(12, 90) = 6.38$, $P < 0.0001$], and dose × time [$F(12, 90) = 5.65$, $P < 0.0001$] effects. The ANOVA dose × time effects were significant for the latency of N1 and N2 [both Fs$(2, 51) > 10.19$, both Ps < 0.0001]. Recovery ratios for the FEP did not show significant dose [$F(9,$

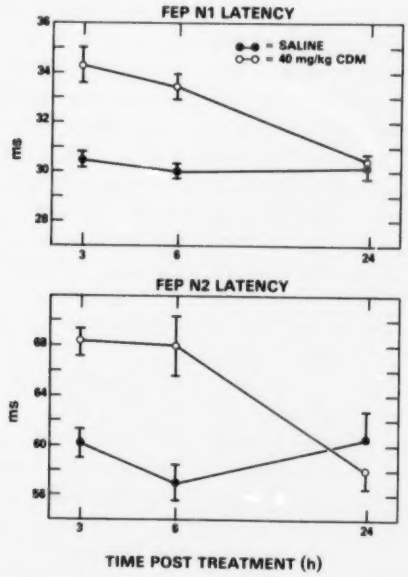

FIG. 7. Time course of CDM action on selected FEP latency and amplitude values. Other measured variables showed a similar temporal pattern. The time course of FEP changes resembled that of the PREP changes, being slightly diminished at 6 h compared with 3, and normal at 24 h after administration of CDM.

24) = 2.51, $P > 0.0345$], time [$F(18, 84) = 1.27$, $P > 0.23$], or dose × time [$F(18, 84) = 1.56$, $P > 0.09$] effects.

DISCUSSION

Acute administration of CDM produced a number of changes in visual evoked potentials. Most prominent was a dose-related increase in PREP N1P1 and P1N3 peak-to-peak amplitudes. The amplitudes for the 40-mg/kg groups were more than 200% of control values, and represent unusually large amplitude increases in evoked potential research. The amplitude effect was quite selective—PREP amplitudes increased but FEP amplitudes were unchanged. The lack of effect in FEP amplitudes cannot be attributed to a lack of sensitivity of the measure, because other treatments such as anesthesia combined with hypothermia produce readily detectable FEP amplitude increases (10). Amplitude alterations produced by CDM are not unique to the visual system as increases in the amplitude, as well as the latency, of click-elicited brain-stem auditory evoked potentials also occur (20).

The stimuli used to elicit the FEPs and PREPs differed on many dimensions. The FEP-eliciting stimulus was a grossly artificial stimulus. It was a brief, high-intensity strobe flash, devoid of pattern or contrast, and presented against a background of very different illumination. On the other hand, the PREP-eliciting stimulus was the phase reversal of a steady, patterned stimulus, it was of high contrast, of lower overall illumination than the FEP stimulus, and featured no abrupt changes in illumination. Because mammalian visual systems possess elaborate mechanisms for encoding the finely patterned, fairly evenly illuminated visual world, there must be functions of the visual system which are untested by flash stimuli, but disrupted by CDM. At this point it is impossible to determine which of the stimulation differences was responsible for the selective effect of CDM on PREP but not FEP amplitudes.

Not all PREP peaks were increased in amplitude by CDM exposure. Component N2P2 diminished to noise levels at the lowest dose, 5 mg/kg. Though this change was not significant when probability values were Bonferroni protected, the variability of N2P2 amplitude was large in relation to its magnitude making statistical significance difficult to attain. Nevertheless, the loss of this component was an apparent consequence of CDM exposure. Whether the potentials that create peaks N2 and P2 had ceased to be generated, or the small voltages were merely swamped by the large increase in P1 is unclear.

Unlike the selective amplitude increase in PREPs but not FEPs, CDM increased the latencies of peaks evenly in both responses. Large, reliable dose-related increases in the peak latencies of most of the measured peaks were produced by CDM. In general, among the peaks affected, the amount of peak latency increase was directly related to the normal peak latency. The uniform nature of the latency increase across both PREP and FEP peaks suggests a common mode of action, unlike the selective peak amplitude effect of CDM. Alternatively, PREP latency increases could result from the large amplitude increases. Subsequent experiments have shown that CDM-induced hypothermia may be responsible for most, but not all, of the peak latency increases. Unlike the latency increases, the peak amplitude increases were independent of body temperature, suggesting a dual mode of CDM action (Boyes *et al.*, in preparation).

The FEP recovery ratios were unaltered by CDM exposure. In a paired-flash procedure, the second stimulus elicits a response in the wake of ongoing excitatory and inhibitory activity from the first response (11). That CDM did not alter FEP recovery ratios or FEP amplitudes suggests that a general shift in neuronal excitation did not occur. This is additional evidence that the PREP amplitude increases were not a result of widespread changes but were of a selective nature.

A single injection of CDM produced rather brief effects. Evoked potential amplitudes and latencies returned to control values between 6 and 24 h after administration of the agent. Behavioral studies show a comparably short duration of CDM action (27). The finding that the evoked potential changes returned to normal within 24 h indicates that CDM acted through rapidly reversible mechanisms. The pathway through which CDM altered evoked potentials remains to be determined. Recent investigations suggest that monoaminergic systems may be sensitive sites of CDM action (17). CDM is rapidly broken down in rats to the mono- and di-*N*-demethyl metabolites (22, 23). The *N*-demethyl metabolites of CDM are potent octopamine agonists in insects (14, 18, 30) acting to stimulate α-adrenergic receptors. Robinson and Bittle (38) showed *N*-demethylchlordimeform to be a partial agonist and antagonist of α-adrenergic receptors in rabbit ear arteries *in vitro*. Mammalian visual function is dependent on monoaminergic neurotransmission. Thus, the monoaminergic systems are attractive candidates for potential sites of action. Perhaps CDM, or its *N*-demethyl metabolites, could act on adrenergic receptors in the rat visual system to produce the selective visual evoked potential changes. Hsu and Kakuk (19), however, failed to produce α_2-adrenoreceptor-mediated changes in the heart rate or pupil diameter of rats after i.v. administration of CDM. In addition, Onofrj and Bodis-Wollner (33) saw amplitude reductions in the PREPs and FEPs of rats treated with α-methyl-*p*-tyrosine (a tyrosine hydroxylase inhibitor), but no large amplitude changes followed apomorphine (a dopamine agonist) or haloperidol (a dopamine antagonist). Dyer *et al.* (12) produced amplitude reductions in the FEPs of rats with α-methyl-para-tyrosine, and an amplitude increase of FEP component P3N3 after haloperidol treatment, yet FEP amplitudes were unchanged by CDM. Treatment with the monoamine oxidase inhibitor, pargyline, does not produce evoked potential alterations similar to CDM (Boyes *et al.*, in preparation). These findings must necessarily temper the scope of monoaminergic hypotheses of CDM action in the rat visual system.

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